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REVIEW ARTICLE

The virulence plasmids of *Salmonella*

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Summary Certain *Salmonella* serovars belonging to subspecies I carry a large, low-copy-number plasmid that contains virulence genes. Virulence plasmids are required to trigger systemic disease; their involvement in the enteric stage of the infection is unclear. *Salmonella* virulence plasmids are heterogeneous in size (50–90 kb), but all share a 7.8 kb region, *spv*, required for bacterial multiplication in the reticulo-endothelial system. Other loci of the plasmid, such as the fimbrial operon *pef*, the conjugal transfer gene *traT* and the enigmatic *rck* and *rsk* loci, may play a role in other stages of the infection process. The virulence plasmid of *Salmonella typhimurium* LT2 is self-transmissible; virulence plasmids from other serovars, such as *Salmonella enteritidis* and *Salmonella choleraesuis*, carry incomplete *tra* operons. The presence of virulence plasmids in host-adapted serovars suggests that virulence plasmid acquisition may have expanded the host range of *Salmonella*.

Key words *Salmonella* · Virulence plasmid · Bacterial adhesion · Serum resistance · Systemic infection

Introduction

Salmonella is a facultative intracellular pathogen that causes a variety of infectious diseases. The most common of such diseases is gastroenteritis, with bacterial multiplication in intestinal submucosae and diarrhea, caused by the inflammatory response and perhaps also by toxins. In specific hosts, adapted salmonellae produce systemic diseases such as typhoid and paratyphoid fevers in humans. If host defenses are impaired, as in elderly or AIDS patients, *Salmonella* can enter the bloodstream and cause septicemia, which is often fatal [47].

A recent proposal based on molecular relationships classifies *Salmonella* into two species, *Salmonella enterica* and *Salmonella bongori* [40]. In turn, *S. enterica* is divided into seven phylogenetic groups, subspecies I, II, IIIa, IIIb, IV, VI and VII. Subspecies I includes 1,367 serovars, some of which are commonly isolated from infected birds and mammals, including humans. The other subspecies mainly colonize cold-blooded vertebrates. Following common practice, we will name the serovars as if they were species, i.e. *Salmonella typhi* rather than *S. enterica* ser. typhi.

Virulence factors responsible for pathogenicity in enteric bacteria are often encoded by plasmids, as in *Escherichia coli*, *Yersinia* spp. and *Shigella* spp. In *Salmonella*, the existence of plasmid-borne virulence genes was first suggested in 1982, but current evidence suggests that the contribution of virulence plasmids to pathogenesis in *Salmonella* is less important than in the aforementioned bacteria. Virulence plasmids have been found in only a few serovars of *Salmonella* belonging to subspecies I, particularly those showing host adaptation (Table 1). These plasmids are 50 to 90 kb in size, and have been called "serovar-specific plasmids" (see refs. [7, 20, 21] for reviews). Not every isolate of a plasmid-bearing serovar carries the virulence plasmid [11].

The virulence plasmid of *Salmonella* is important for bacterial multiplication in the reticulo-endothelial system of warm-blooded vertebrates. Only a 7.8 kb region, *spv* (*Salmonella* plasmid virulence), is necessary to confer the virulent phenotype. Other loci of the plasmid, involved in the biosynthesis of fimbriae and in serum resistance, may play a role in other stages of the infection process [20, 21]. *Salmonella typhi*, which causes typhoid fever in humans, and other serovars less often involved in similar human diseases (paratyphoid fever), such as *Salmonella paratyphi A*, *Salmonella paratyphi B*, and *Salmonella sendai*, lack virulence plasmids (and the *spv* region). *Salmonella paratyphi C* is an exception, but the contribution of its virulence plasmid to systemic diseases is unknown [7, 11]. The importance of the virulence plasmid in septicemic diseases caused by non-typhoid serovars can only be inferred from indirect evidence: plasmids are more frequently found in *Salmonella typhimurium* and *Salmonella enteritidis* strains

Table 1 *Salmonella* serovars carrying virulence plasmids[1]

Serovar	Host	Disease	Plasmid size (kb)
S. paratyphi C	Humans	Paratyphoid fever	54
S. enteritidis	Rodents	Murine typhoid	60
S. typhimurium	Rodents	Murine typhoid	90
S. dublin	Cattle	Septicemic disease	80
S. choleraesuis	Pigs	Septicemic disease	50
S. gallinarum[2]	Poultry	Fowl typhoid	85
S. pullorum[2]	Poultry	Pullorum disease	85
S. abortusovis	Sheep	Abortion	50–67

[1] Other serovars of *Salmonella* that could also bear virulence plasmids, as deduced from hybridization experiments, are *S. johannesburg, S. kottbus, S. give, S. newport* and *S. derby* [7, 11, 20, 21, 29].
[2] *S. gallinarum* and *S. pullorum* are biovars of the same serovars.

isolated from blood and other extraintestinal sources than in strains isolated from feces. The differences in frequency reported are from 76% vs 42% [17] to 98% vs 68% [34].

In most cases, the role of the virulence plasmid has been evaluated in experimental mouse infections involving either oral or intraperitoneal administration, but assays in natural hosts (see Table 1) have also been performed [7, 20, 21]. The presence of the plasmid decreases 50% lethal doses by a factor of 10 to 10^6, depending on the serovar and the route of inoculation. There is general agreement that the plasmid is not involved in the enteric stage of the disease [56]. However, a recent study in calves, the natural host for *Salmonella dublin*, revealed that the virulence plasmid affects the severity of the enteric disease [32].

The exact role of the virulence plasmid in pathogenesis is unclear. Evidence exists that *spv* genes enable *S. typhimurium* to infect the spleen and the liver by increasing the rate of bacterial replication within host cells [22]. Plasmid-cured strains are able to colonize and persist in the spleen and the liver, but bacterial growth is controlled by host defenses and infection does not develop. There is controversy, probably caused by experimental differences, about the effect of the virulence plasmid on the proliferation of *Salmonella* in macrophages. Studies of the effects of cytokines IFN-γ and TNF-α on strains with and without the virulence plasmid have also produced conflicting results. Muotiala and Mäkelä [35] reported increases by factors of 1000 and 100, respectively, in the counts of plasmid-cured *S. typhimurium* and *S. enteritidis* isolated from the liver of *Ity*^{S} mice treated with an anti-IFN-γ monoclonal antibody. In contrast, Gulig et al. [23] observed that a *S. typhimurium* strain lacking the virulence plasmid behaved like the wild type upon infection of the spleen of cytokine-depleted mice, and concluded that the function of the virulence plasmid is not to overcome the inhibition of bacterial replication mediated by IFN-γ and TNF-α. The discrepancy was attributed to the differences in the route of inoculation, intravenous in the first case and oral in the second, which might lead to the colonization of different cell populations. The virulence plasmid affects intracellular growth in macrophages, but not in non-phagocytic cells [24].

Maintenance and transfer functions

Two independent replicons, *repB* and *repC*, have been identified in the virulence plasmids of *S. enteritidis* and *S. typhimurium*. Both replicons are functional and control the low copy number of these plasmids (Figs. 1 and 2) [44, 51]. Both have also been detected in the *S. choleraesuis* plasmid, whereas the plasmids of *S. dublin, S. gallinarum* and *S. pullorum* seem to carry only *repB*, as indicated by hybridization experiments [50]. Of these plasmids, only those of *S. gallinarum* and *S. pullorum* are compatible with the *S. typhimurium* virulence plasmid [37].

The entire *repB* and *repC* replicons of the *S. enteritidis* plasmid and fragments from the replicons of the *S. typhimurium, S. dublin*, and *S. choleraesuis* plasmids have been sequenced and compared with those of other enterobacterial plasmids, with accession numbers: AF120497, AF120495, AF120498 [13a, 44]. The *repB* replicon is very similar to the RepFIIA replicon of the virulence plasmid pYVe439-80 of *Yersinia enterocolitica* (81% identity in nucleotide sequence). Its replication protein, RepA, is identical to that of the *S. choleraesuis* plasmid, and almost identical (99%) to those encoded by the plasmids of *S. typhimurium* and *S. dublin*. The RepFIIA replicons share homologous regions separated by non-homologous stretches, and a phylogenetic classification based on the similarity (>95%) of the homologous regions has been proposed [33]. According to this classification, the virulence plasmids of *Salmonella* do not belong to any of the established groups [44]. In the case of *repC*, we estimated 98% identity to the *repC* replicon of the *S. typhimurium* plasmid, and 90% identity to the enteroadherent factor (EAF) plasmid of *E. coli*, which encodes bundle-forming pili (BFP). Interestingly, the chromosomal genes encoding BFP in *Salmonella* are homologous to those of the EAF plasmid [49]. The *repC* replicon shows the typical organization of the RepFIB family, with a set of direct repeats (iterons) that exert stringent control of copy number (Fig. 2). The first iterons (B, C, and D) correspond to the reported sequence of the *rsk* fragment, which has been associated with the control of serum resistance [55].

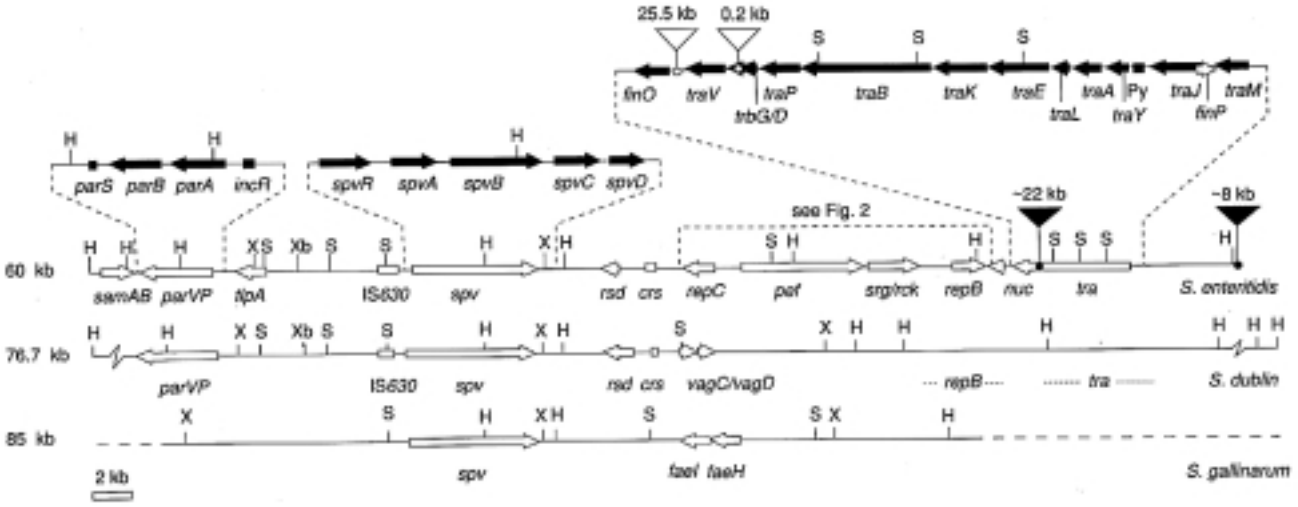

Fig. 1 Genetic organization of the virulence plasmids of several serovars of *Salmonella*. The alignment of the maps of *S. dublin* and *S. gallinarum* plasmids is tentative [31, 46]. The *par*, *spv* and *tra* regions are shown in detail above the *S. enteritidis* plasmid map. The *S. enteritidis* plasmid differs from the *S. typhimurium* plasmid by only two deletions, the location and estimated sizes of which are indicated (black triangles). In turn, the deletions detected in the *tra* region of the *S. enteritidis* plasmid with respect to the F plasmid are shown as white triangles [12, 44]. Restriction-site abbreviations are as follows: H, *Hin*dIII; S, *Sal*I; X, *Xho*I; Xb, *Xba*I

Despite their low copy number (1–2 copies per chromosome), *Salmonella* virulence plasmids are very stable, and several regions responsible for partition functions have been characterized. The *parVP* locus, initially described as a replication region, *repA*, expresses partition and incompatibility functions [50]. It actually comprises two partition systems: one requires the ParA and ParB proteins and the *parS* locus, whereas the other requires ParA and the *incR* locus (Fig. 1) [13]. The *parVP* region is present in the plasmids of *S. enteritidis*, *S. typhimurium* and *S. choleraesuis*, but not in those of *S. dublin*, *S. derby* and *S. pullorum* [11]. The stability of the *S. dublin* plasmid depends on a multimer resolution system that consists of a resolvase, encoded by the *crs* gene, and a resolution site, *rsd*. This system is also present in other *Salmonella plasmids*. A locus called *vagC/vagD* may also be involved in the maintenance of the *S. dublin* plasmid, delaying cell division until replication has been completed [39]. This locus is absent from other plasmids [12].

The virulence plasmids of *Salmonella* have been usually classified as non-conjugative. However, a recent report indicating that the virulence plasmid of *S. typhimurium* LT2 is self-transmissible [3] may prompt reconsideration of this issue. Some virulence plasmids appear to contain a more or less complete *tra* operon, whereas others have suffered major deletions as in the case of the *S. enteritidis* plasmid (Fig. 1) [44]. The *S. choleraesuis* plasmid contains the *traT* locus, but has probably lost most of the operon, and the plasmid of *S. dublin* lacks homology with the *tra* region. The presence of *tra* operons (complete or incomplete) in most virulence plasmids suggests that a *Salmonella* ancestor acquired the virulence plasmid by conjugation, and that divergence has occurred during the evolution of the various serovars. Boyd and Hartl [10] have drawn attention to the high level of similarity between the *tra* genes of the F-related virulence plasmids of *Salmonella* and those of the F episome of *E. coli*. This view was confirmed by sequencing of a 4.6 kb region from the *S. typhimurium* LT2 virulence plasmid, pSLT, accession number AJ011572 [52]. Open-reading-frames homologous to the *traM*, *traJ*, *traY*, *traA*, *traL*, *traE*, *traK* and *traB* genes of F are present in pSLT. A

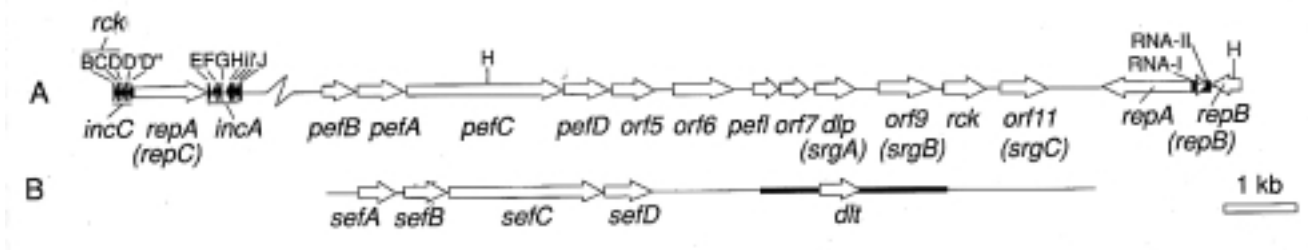

Fig. 2 (A) Genetic organization in the *S. enteritidis* and *S. typhimurium* plasmids around the *pef* locus. (B) The only region of the chromosome of *S. typhi* with homology to the plasmid of *S. enteritidis* (thick line) and the adjacent region carrying *sef* loci [43]

putative *finP* gene is also present, overlapping with *traJ*, an arrangement identical to that found in F [52].

The *spv* region

The *spv* locus, when cloned in a low-copy number vector, is sufficient to restore the virulence phenotype of a plasmid-cured strain [21]. The location of the *spv* region in plasmids carried by isolates of subspecies I contrasts with the presence of sequences hybridizing to an *spv* probe on the chromosome of all isolates belonging to the *Salmonella* subspecies II, IIIA, and VII, and in 2 of 9 strains of subspecies IV [11].

The *spv* region of subspecies I harbors five genes, *spvRABCD*, organized as a regulon and well conserved among serovars (Fig. 1). Sequencing of *spv* genes has provided no clues about their function, except in the case of SpvR, a 33-kDa polypeptide homologous to the LysR family of regulatory proteins (see refs. [20, 21] for reviews). The middle portion of the SpvB sequence shows a certain degree of similarity to the Ace toxin of *Vibrio cholerae* (Accessory cholera enterotoxin), which acts as an ion transporter across cell membranes and contributes to diarrhea [53].

There is evidence that SpvR binds to an operator region of the *spvA* promoter in a two-step fashion. Binding of SpvR to a promoter-distal regulatory sequence favors subsequent binding to a proximal region, which is essential to activate *spvA* transcription. Expression of *spvR* is self-regulated, and the structure of its promoter is similar to that of *spvA*. A weak interaction with SpvR seems to favor *spvR* expression, whereas a stronger interaction is required to activate the *spvA* promoter [48].

The *rpoS* gene, which encodes the alternative sigma factor σ^s, also regulates the expression of *spv* genes. Expression of *rpoS* is induced after entry of *Salmonella* into macrophages or epithelial cells, or in vitro during the stationary growth phase[15]. Further regulation is mediated by the nucleoid-associated protein H-NS [42]. Expression of the *spv* genes in response to intracellular signals supports the view that the virulence plasmid may play a role in the multiplication of *Salmonella* as an intracellular parasite.

The synthesis and localization of the proteins encoded by the four *spv* structural genes have been studied using specific antibodies [16]. SpvA (28 kDa) is found exclusively in the outer membrane. It must be exported by a system other than the general secretory pathway, because it lacks a typical bacterial signal peptide. Surprisingly, *spvA* mutations do not reduce the virulence of *S. dublin* in mice infected by the intraperitoneal route.

SpvB (66 kDa) is found in two fractions, and the largest fraction is cytoplasmic. Small amounts of SpvB are found in the inner membrane. Thus SpvB may have a transmembrane-associated function. SpvB is absolutely essential for virulence, as shown by the fact that *spvB* mutants are avirulent in mice [45].

SpvC (28 kDa) is only detected in the cytoplasm, whereas SpvD (25 kDa) is exported outside the cell (although small amounts are found in various cellular fractions). Again, nothing is known of the mechanism of SpvD export, as the polypeptide is not cleaved and has a hydrophilic N-terminus. The function that SpvD could fulfill outside the cell is unknown. Mutations in *spvC* and *spvD* genes cause various (allele-specific) defects in *Salmonella* virulence.

Plasmid-encoded fimbriae (PEF)

The discovery of a cluster in the *S. typhimurium* plasmid for the biosynthesis of fimbriae was the serendipitous result of a search for genes able to complement a defect in cobalamin uptake. Overexpression of one cloned gene affected outer membrane integrity, causing leakage of cobalamins. The putative protein encoded by the cloned gene was very similar to PapC and FaeD, two outer membrane proteins involved in the export and assembly of Pap and K88 fimbriae in *E. coli* [41].

The *pef* (plasmid encoded fimbriae) locus contains four genes (*pefBACDI*) named after the homology of their products with those of other fimbrial operons, and additional ORFs (*orf5*, *orf6*, *orf7*, *orf8*, *orf9*, and *orf11*) whose function cannot be deduced from sequence analysis (Fig. 2). Surprisingly, the known gene *rck*, involved in serum resistance, was found between the last two ORFs. Using transcriptional fusions to *lacZY*, Ahmer et al. [2] found that, in *S. typhimurium*, *orf8*, *orf9*, *rck*, and *orf11* are regulated by a chromosomal gene homologous to *sdiA* of *E. coli*, and renamed these ORFs *srgA*, *srgB*, and *srgC* (*sdiA*-regulated genes). In *E. coli*, SdiA is a quorum sensor, but the role of its homolog in *S. typhimurium* has not been established.

In both *E. coli* and *S. typhimurium*, *pef* genes carried on a multicopy plasmid determine the formation of surface filamentous structures. Transposon insertions in *pefA*, *pefC*, *orf5* and *orf6*, but not in *orf8* (*srgA*), abolish the formation of fimbriae [18]. A locus homologous to *pef* has been cloned from the *S. enteritidis* plasmid and expressed in *E. coli* (but not in *S. enteritidis*). The similarity between the *pefA* genes of *S. enteritidis* and *S. typhimurium* plasmids is 76% in nucleotide sequence, and 82% in the deduced amino acid sequence [57].

In vivo assays have shown that the *pef* genes are expressed by both *S. enteritidis* and *S. typhimurium*, as orally infected chickens develop specific antibodies. Using *pefC* insertion mutants of *Salmonella typhimurium*, Bäumler et al. [6] showed that PEF mediate adhesion to the small intestine of mice, and that they are necessary, but not sufficient, to induce fluid secretion in the suckling murine model. Adhesion mediated by PEF is different from that induced by the chromosome-encoded *lpf* (long polar fimbriae), which promote the adhesion of *Salmonella* to Peyer's patches [9]. However, PEF do not mediate adhesion to various human tested cell lines [8].

Sequences homologous to *pef* have been detected by hybridization in at least one strain of *S. choleraesuis* and one of *S. bovismorbificans*, but not in 247 strains of *S. dublin*, all carrying the virulence plasmid. The *S. gallinarum* plasmid does not contain *pef* either, but Rychlik [46] has characterized two ORFs homologous to *faeH* and *faeI* (>70% identical at the nucleotide level). The *faeH* and *faeI* genes of *E. coli* encode subunits of K88 fimbriae. Probes derived from the *S. gallinarum* ORFs hybridized to the plasmids of *S. pullorum* and *S. dublin*, but not to those from other salmonellae [46].

We screened for sequence similarity between fragments from the *S. enteritidis* plasmid and the chromosome of *S. typhi*, and found that the only positive signal was obtained with a probe for the *pef* region. The corresponding region from *S. typhi* was cloned and sequenced, and was 70–98% similar to the ORFs *pefI*, *orf7*, *orf8*, and *orf9* of the *S. enteritidis* plasmid. Upstream from these ORFs, a region is found with a high degree of similarity (96–97%) to the chromosomal *sef* loci of *S. enteritidis*, which encode SEF14 and SEF18 fimbriae [43]. The highest degree of sequence similarity between the chromosome of *S. typhi* and the virulence plasmid of *S. enteritidis* corresponds to *orf8* (*srgA*). The plasmid-borne locus encodes a putative thiol:disulfide oxidoreductase similar to DsbA from *E. coli*, and has been named *dlp* (*dsbA*-like plasmid-encoded). Its expression in *E. coli* complements the pleiotropic phenotype of *dsbA* mutants. In contrast, the allele from the *S. typhi* chromosome (*dlt*) is inactive if expressed in *E. coli*, although its product can be detected in the cytoplasm. This absence of activity has been attributed to a point mutation which blocks processing and export of the protein to the periplasm. A sequence homologous to *dlp* has been detected in the chromosome of *S. enteritidis*, but not in that of *S. typhimurium* [43]. The enzymes of the DsbA family are required for the secretion and correct folding of various proteins, including virulence factors of *E. coli*, *Vibrio cholerae* and *Shigella flexnerii* (see Ref. [4] for a review). The *dlt* gene is not the only non-functional fimbrial gene of *S. typhi*: *sefA* and *sefD*, which encode structural subunits of fimbriae SEF14 and SEF18 respectively, both present frameshift mutations. The same single-base insertion has been observed in the sequence of *S. typhi* published by the Sanger Center and in two clinical isolates (Serrano and Rotger, unpublished results).

The fimbrial genes *pef* and *sef* may have been acquired recently by *Salmonella*, as they are found only in certain phylogenetically related strains, rather than in specific serovars. Each acquisition may have occurred by an independent event of horizontal transfer, as indicated by the G + C contents of *pef* and *sef* (e.g. *sef* has a G + C content of only 35.2% vs the 52% typical of the *Salmonella* chromosome). The *pef* and *sef* loci share a high degree of homology and a similar organization. The acquisition of these related types of fimbriae may have enabled *Salmonella* to adapt to different situations of colonization. Quoting Bäumler [5], "the acquisition of different fimbrial operons may have been one of the mechanisms by which *Salmonella* serovars were able to expand their host range to include many domesticated animals". The presence of K88-related sequences in *S. gallinarum*, *S. pullorum* and *S. dublin*, as opposed to the presence of *pef* in *S. enteritidis*, *S. typhimurium*, and *S. choleraesuis*, strengthens the idea that different serovars have adapted to different hosts. The adaptation process may also have involved the selection of mutants that have lost some virulence determinants; this may be the case for SEF14 and SEF18 in *S. typhi*.

Resistance to serum

The role of the virulence plasmid in the resistance of *Salmonella* to the complement-mediated bacteriolytic activity of normal serum remains controversial. One reason is that serum resistance mediated by the long-chain lipopolysaccharide (LPS) of *Salmonella* can mask other mechanisms [20]. Aside from this caveat, three virulence plasmid genes have been suggested to be involved in serum resistance: *traT*, *rck* and *rsk*.

TraT, a 27 kDa protein encoded by the transfer region of the *S. typhimurium* plasmid, confers weak serum resistance. TraT proteins encoded by F and other conjugative plasmids have a similar effect. The contribution of *traT* to the serum resistance of *S. typhimurium* is unclear [20]. Glöckner et al. [19] found that *traT*-mediated resistance is detectable in *S. typhimurium* carrying freshly transferred plasmids, but disappears after 20 generations, and is effective only against the alternative pathway. The *traT* gene is absent from the plasmids of *S. enteritidis* [12], *S. dublin* and *S. choleraesuis* [19].

The *rck* gene has been detected on the virulence plasmids of *S. typhimurium* [25] and *S. enteritidis* [12], downstream from the *pef* operon (Fig. 2). Expression of *rck* is regulated by a SdiA analog [2]. Expression of *rck* confers high-level serum resistance both in *S. typhimurium* and *E. coli*, independent of LPS structure. Furthermore, *rck* confers to *E. coli* the ability to invade cultured mammalian cells [27]. The Rck product is a 19 kDa precursor that is inserted in the outer membrane after cleavage of a leader sequence, and inhibits the polymerization of the C9 protein of complement [26].

Rck is a member of a family of outer membrane proteins that includes other proteins involved in virulence, such as Ail (encoded by a chromosomal gene of *Yersinia enterocolitica*) and PagC (encoded by a *phoP*-regulated gene in the chromosome of *S. typhimurium*). Other members of the family are OmpX of *Enterobacter cloacae* and the Lom product of bacteriophage lambda (which has no known connection with virulence). Only Rck and Ail share the ability to induce both serum resistance and epithelial cell invasion, whereas PagC appears to be involved in the survival of *S. typhimurium* in macrophages [27]. All these proteins present alternating hydrophobic and hydrophilic regions. It has been suggested that they fold across the outer membrane, with four extracellular

loops corresponding to the hydrophilic stretches, and with the hydrophobic segments inserted in the membrane. Mutants carrying a single amino acid change in the proposed third outer loop of Rck are affected in both serum resistance and cell invasion. A chimeric Rck-PagC protein with the first two loops of Rck and the third and fourth of PagC is likewise unable to confer serum resistance and cell invasion ability, whereas a Rck-PagC hybrid with only the fourth PagC loop has similar activity to wild-type Rck [14].

Vandenbosch et al. [54] observed that integration of the virulence plasmid in the bacterial chromosome increased the susceptibility of smooth strains of *S. typhimurium* to decomplemented serum. The subsequent introduction of an autonomous copy of the plasmid restored, and even increased, serum resistance. In a screen for cloned sequences able to complement the serum resistance defect of a *S. typhimurium* strain with the virulence plasmid integrated, these authors found a small fragment (66 bp) that restored the wild-type phenotype if provided in multiple copy. As the cloned fragment did not confer serum resistance to *E. coli* or *S. typhimurium* lacking the virulence plasmid, the authors suggested that *rsk* regulated genes located on the plasmid, on the chromosome or on both elements, and named this short stretch *rsk* (regulation of serum killing) [55].

Integration of the plasmid into the *S. typhimurium* chromosome not only increases susceptibility to serum, but also the lag time of cultures grown in minimal medium. The introduction of an autonomous copy of the virulence plasmid restores the normal lag time, as does a multicopy vector carrying the *rsk* sequence. These observations indicate that integration of the virulence plasmid may affect chromosome replication, perhaps through the activity of the plasmid replicons.

The only conserved region shared by the plasmids of *S. enteritidis* and *S. typhimurium* and the chromosomes of *S. enteritidis* and *S. typhi* is located around the *dlp* (*srgA*) gene (including *pefI*, *orf7*, and *orf9* [*srgB*]). This conserved region is also found in *S. typhimurium*, which lacks a *dlp* homolog, and in other serovars such as *S. choleraesuis* and *S. dublin* [29, 43]. This region is a good candidate for RecA-mediated integration of the virulence plasmid into the chromosome. As promoter sequences have not been found upstream from *rck* [25], the expression of this gene probably depends on the upstream *srg*AB genes. It is thus conceivable that homologous recombination affects *rck* expression, impairing serum resistance upon integration of the virulence plasmid.

As indicated above, the *rsk* sequence is part of the *repB* replicon of the *S. enteritidis* plasmid, and corresponds to the B, C, and D iterons of the *incC* locus of the RepFIB replicon. This finding is consistent with the suggestion of Vandenbosch et al. [55] that *rsk* is a regulatory element able to bind the replication protein, RepA. However, the mechanism of copy number control and incompatibility of RepFIB replicons is not completely understood. Studies carried out with the replicon of the P1 plasmid favor a model in which the daughter plasmid molecules interact via the RepA protein bound to the iterons of two plasmids (handcuffing). In this state, replication is inhibited and does not restart until the plasmids segregate [1]. However, there is also evidence that the presence of *incC* (without *incA*) in a multicopy plasmid can titrate the RepA protein and inhibit replication [38]. The introduction of *rsk* in a multicopy vector might affect the integrated copy of the virulence plasmid and restore the normal replication of the chromosome (if this were the reason for the increased lag time). However, this hypothesis does not explain the recovery of the serum-resistant phenotype.

Other loci

A number of loci with unknown funtions are found on *Salmonella* virulence plasmids (Fig. 1); the most intriguing is probably *tlpA*, found in the plasmids of *S. typhimurium* [30] and *S. enteritidis* [44]. Its product, TlpA, is a long coiled-coil protein that acts as a thermosensor, repressing its own expression when temperature increases. A motif present in the *tlpA* promoter is also found in certain virulence-related genes, both on the virulence plasmid (*spvA*) and on the chromosome (*pagC*, *prgH* and *orgA*) of *Salmonella*, but no evidence exists that any of these genes are regulated by TlpA [28]. Other loci of unknown function include *samAB*, homologous to *umuDC* [36], and the *nuc* locus, which bears homology to an endonuclease gene of pKM101. Upstream from *spv*, a sequence related to IS*630* has been detected, but nothing is known about its activity [20, 44].

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